Pharmacology Biochemistry & Behavior, Vol. 7, pp. 135–138.

Visual Evoked Response and Behavioral Correlates of Plasma Methadone Concentrations in Cats[1,2]

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(Received 21 March 1977)

SNYDER, E. W., D. E. SHEARER, R. E. DUSTMAN AND E. C. BECK. *Visual evoked response and behavioral correlates of plasma methadone concentrations in cats.* PHARMAC. BIOCHEM. BEHAV. 7(2) 135–138, 1977. – Twelve cats were implanted with cortical and depth electrodes. After they recovered from the operation, visual evoked responses (VERs) were recorded at 20 min following saline and methadone (0.5, 1, 2, 3, 4 mg/kg) administered IP in a semi-random order to each cat. Four other cats were similarly drugged and plasma was obtained for radioimmunoassay of methadone content. At the three lowest dose levels behavioral excitation and salivation were evident in some cats, while their VER configuration remained essentially unaltered and plasma methadone was barely measurable. Following the 3 and 4 mg/kg doses plasma methadone concentration increased dramatically, behavioral excitation and salivation were evident in most cats and VERs were reliably altered. The VER alterations, consisting of amplitude attenuation and a decrease in some latencies, were restricted to secondary VER components occurring between 50 and 100 msec despite the animals' extreme behavioral excitation. These results suggest that the reticular formation is not a principal site of the drug's effect.

Visual evoked responses Methadone plasma levels Feline methadone mania

CATS and some other mammals, e.g., certain ungulates such as horses and goats, respond to moderate doses of most opioids with behavioral excitation rather than the sedative effects generally observed in man and other mammals [15]. The EEG correlates of this feline mania have been carefully studied as have the electrophysiological correlates of narcotic analgesia in general (for review, see [5,11]). However, despite the proven utility of somatosensory, auditory and visual evoked responses (VERs) in drug research (e.g., [6, 8, 21]) apparently only one attempt has been made to study the opioid-manic cat using these techniques. Sinitsin [22] reported that high doses of morphine enhanced most components of auditory and visual evoked responses (VERs) in cats, while somesthetic evoked responses were reduced regardless of dose. The author attributed these effects to selective changes in cortical and thalamic excitability. Unfortunately this study only compared low (1–3 mg/kg) and high (5–10 mg/kg) doses of morphine in cats immobilized with succinylcholine. Data were presented for only 10 evoked responses recorded from a single cat prior to and at an indeterminate time following injection, and no behavioral data were given.

In humans [9] a preanesthetic dose (0.13 mg/kg) of morphine was found to have no systematic effect on the VER, while a more recent paper [14] described a time-dependent, biphasic effect of l-α-acetylmethadol with initial enhancement of secondary VER components followed by suppression in rabbits. Although no consistent interspecies pattern emerges from these studies it has been hypothesized [5] that primary components of the evoked response are quite resistant to opioids while secondary components may be either depressed or enhanced. We have, however, observed a substantial alteration of all VER components in behaviorally depressed monkeys intoxicated by heightened plasma-methadone concentrations [23]. Therefore, we have expanded our investigation in an attempt to determine the effects of a wide range of doses in a species (cats) whose behavioral response to opioids is quite unlike that of primates [2].

METHOD

Animals

Seven male and five female cats were used in the evoked response study. Four additional cats, two of each sex, were used to assess methadone plasma levels. All animals were tractable and in good health. Body weights ranged from 3 to 5 kg; ages ranged from 2 to 4 years.

[1] Supported by the Medical Research Service of the Veterans Administration, Project No. 0864–05, and by the U.S. Public Health Service Research Grant DA–00388 from the National Institute of Mental Health. The authors wish to thank Mr. Paul Sine and the employees of the Animal Research Facility, with special acknowledgement to Colonel Underwood for his expert assistance in the handling of the experimental animals.

[2] This paper was presented in preliminary form at the National Drug Abuse Conference, San Francisco, CA, May 1977.

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Surgical Procedures

Cats, anesthetized with pentobarbital sodium (30 mg/kg IP), were stereotaxically implanted with cortical and subcortical electrodes under sterile conditions. Cortical electrodes, consisting of stainless steel screws, were threaded through the calvarium to make contact with 2 mm^2 of dura overlying posterior marginal and posterior suprasylvian gyri. Subcortical electrodes were inserted into midbrain reticular, thalamic and limbic regions. Only VERs recorded from posterior marginal gyrus will be reported. All electrodes, including the frontal sinus reference, were connected to a miniature amphenol plug attached to the skull with screws and dental acrylic. The animals were allowed two weeks to recover from surgery prior to recording. All experiments involved waking animals with mydriatic pupils (1% Mydriacyl).

Apparatus

All experiments were conducted in a semidarkened and electrically shielded recording room. A Grass model PS22 photostimulator was used to present 10 μsec light pulses into a reflecting hemicylinder which was 100 cm distant. The illumination of the reflecting surface was 20 lux as measured from the position of the cat's eyes. The hemicylinder was placed in front of a hammock in which the animals were held under light restraint. Background white noise at 70 db with reference to 0.0002 μbars was fed into the recording room to mask extraneous sounds. EEG was amplified by a Grass Model 78B EEG/polygraph (bandwidth, 0.3 Hz–300 Hz; time constant, 0.25 sec) and recorded on magnetic tape. The stored EEG was digitized at a rate of 500/sec by an AF01–B analog-digital converter interfaced to a DEC PDP–9 computer which summed and averaged evoked responses. The evoked responses were traced on graph paper by a Complot DP–1 digital plotter.

Procedure

Following postoperative recovery each cat was habituated to the restraining hammock and several baseline recordings were made. Methadone HCL (0.5, 1, 2, 3 and 4 mg/kg) and a saline control were injected intraperitoneally in equal volumes according to an individualized semi-randomized schedule with a minimum of 7 days between treatments. Since we expected 4 mg/kg to be lethal to some cats [2] this dose was administered last. Prior to recording sessions each cat was habituated to the recording room for 15 min and then injected with drug or saline. At fifteen min postinjection the cat was restrained and five min later EEG recording began. Pilot studies had shown that the peak behavioral effect occurred 20 min postinjection. Single photic pulses were presented at 2–3 sec intervals during periods of artifact free EEG. Recording sessions lasted approximately ten min. All cats, well adapted to restraint, remained passive throughout recording despite their obvious behavioral excitation upon release from the hammock. In no case was recording prolonged because of undue muscle artifact. Evoked response to 75 flashes were averaged for each visual evoked response (VER). Drug administration procedures were replicated for four unimplanted cats and, at 20 min past injection, blood was extracted for radioimmunoassay of methadone content. The extraction and assay procedures have been described elsewhere [23].

The behavioral changes which followed methadone treatment are summarized in Table 1. Salivation and loss of righting reflex were obvious and easily scored. Rear leg paralysis was recorded whenever an animal moved by dragging its splayed hind legs [2]. Excitation was recorded when a cat was observed pacing back and forth, running in circles or vigorously attempting to escape. Two behaviors, salivation and excitation were scaled from zero to three depending upon severity. Other behaviors were merely noted for their presence or absence.

Statistical Analysis

VERs plotted for all conditions typically included three positive and three negative components as depicted in Fig. 1. The latency to the peak of each component and peak-to-peak amplitudes were compared across seven treatments (predrug, saline and five doses of methadone) by single factor analysis of variance. Significant individual sources of variation were determined with the use of Duncan's Multiple Range Test [10].

RESULTS

Figure 1 illustrates the typical cat VER and the components which were significantly altered by methadone. Analyses of variance indicated that neither latencies nor amplitudes of the early VER components (P1 and N1) changed significantly as the dose of methadone was increased. However, the amplitude of P2–N2 showed a significant reduction with increasing dose [$F(6,66) = 6.96$, $p<0.001$]. Amplitude at the highest dose was significantly lower than that at the next highest dose as determined by

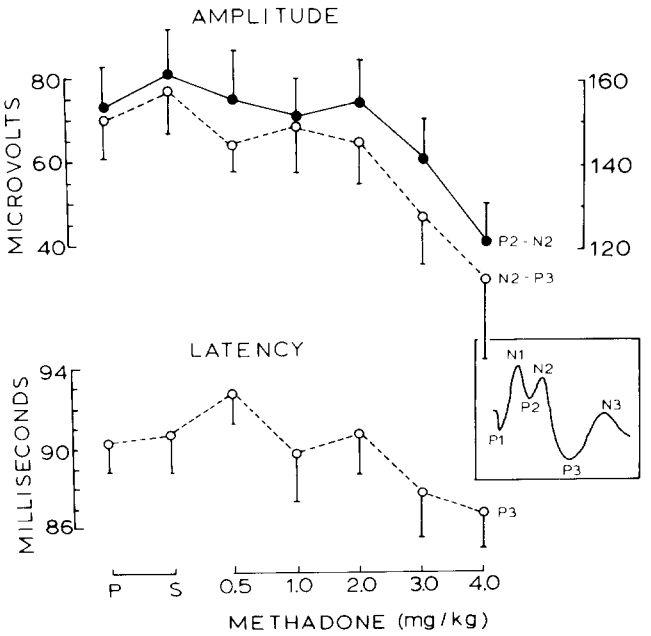

FIG. 1. Mean amplitude and latencies (± SEM) of those VER components modified by increasing doses of methadone. Insert depicts typical VER of the cat. Predrug (P) and saline (S) values are indicated to the left.

TABLE 1

NUMBER OF ANIMALS EVIDENCING VARIOUS BEHAVIORAL CHANGES AT EACH DOSE OF METHADONE

	Excitation	Salivation	Loss of Righting Reflex	Rear Leg Paralysis	Death
Saline	0	0	0	0	0
0.5	1	1	0	0	0
1.0	2	3	0	0	0
2.0	8	10	0	0	0
3.0	16	14	0	0	0
4.0	16	16	5	8	2

Data from implanted and nonimplanted animals are included.

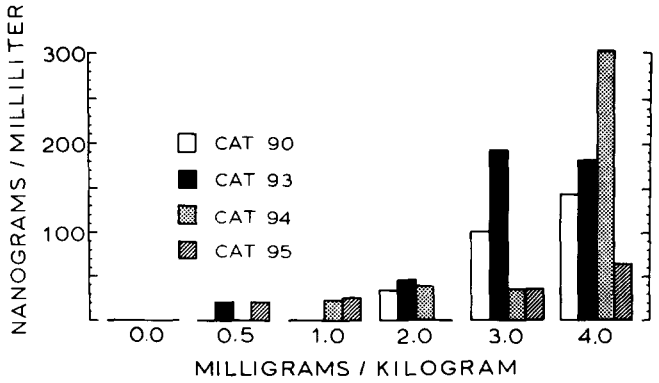

FIG. 2. Plasma methadone concentration (ng/ml) in each of four cats at 20 min past IP injection. Mean values were, in order of increasing dose, 10.2, 12.0, 28.5, 89.8, 171.5 ng/ml. Zero values indicate no detectable methadone in plasma; all cats received every dose.

the Duncan Multiple Range Test ($p<0.01$). The N2–P3 component also showed significant [$F(6,66)=3.51, p<0.01$] reduction in amplitude. However, amplitudes at the two highest doses while differing significantly ($p<0.01$) from values at the low doses, were not significantly different from one another as determined by a posteriori examination. The amplitude of P3–N3 was not reliably altered.

Latencies of the three earliest components (P1, N1, P2) remained remarkably stable across doses. N2 showed a light but nonsignificant decrease with increasing dose while the latency of P3 decreased significantly with increasing dose [$F(6,66)=3.10$, $p<0.01$]. Again the two highest doses were responsible for the significant ($p<0.01$) drug effect.

In short, the amplitude of the P2–N2, a negative wave peaking at about 50 msec, was the most sensitive to the drug's effect while the latency of the succeeding positive wave (P3), decreased significantly at the two highest doses. These VER changes corresponded to dose related increases in the plasma methadone concentration as depicted in Fig. 2. While there was considerable intersubject variability, mean plasma concentrations increased systematically at the two highest doses.

The behavioral effects of methadone are summarized in Table 1. As is apparent, the number of animals evidencing behavioral changes varied directly with the dose. In addition, the severity of excitation and salivation increased dramatically. Thus, correlations between VER parameters (Fig. 1) and these behaviors were in all cases significant and negative (Table 2). No correlations were calculated for the remaining behaviors since they were only scored for their

TABLE 2

CORRELATIONS BETWEEN BEHAVIORAL MEASURES AND VER ACROSS DOSES OF METHADONE

	P2-N2	N2-P3	P3
Excitation	$-.93, p<0.01$	$-.96, p<0.01$	$-.81, p<0.05$
Salivation	$-.88, p<0.01$	$-.95, p<0.01$	$-.86, p<0.05$

Mean amplitudes (P2-N2) and latency (P3) of VER were correlated with those behavioral measures which were scored. Other measures were only noted for their presence or absence. DF for each cell is 5.

presence or absence. In most cases the animals were behaviorally normal at four to five hr past injection. However, those animals evidencing extreme excitation and profuse salivation at the highest doses showed continuing effects for 10 to 12 hr following injection.

DISCUSSION

The results of this study demonstrate that doses of methadone which effect obvious behavioral changes in cats produce changes in selected VER components. The behavioral effects were in many ways similar to those observed previously in one cat [2]. However, while these authors reported extreme behavioral excitation to 3 mg/kg IP, they did not mention salivation which occurred in most of our cats at the two highest doses (Table 1). In addition these authors reported that the animal was "oblivious to external stimuli" [2]. In general, our drugged cats responded to stimulation with increased excitation and vigorous attempts to escape.

The behavioral and VER alterations we observed were substantial only at doses of 3 and 4 mg/kg which produced mean plasma concentrations of 90 and 172 ng/ml, respectively (Fig. 2). In monkeys [23] comparable blood levels ($\geq$130 ng/ml) were occasionally lethal and consistently produced gross behavioral and electrophysiological depression, reflected by attenuation of all VER components, early and late. Thus it would appear that the species-specificity of the behavioral response to methadone can be extended to the drug-induced alteration of the VER.

It has long been generally accepted that the primary components of the VER reflect activity of the classical ascending pathways through specific thalamic nuclei to cortex. (For review of the neurogenesis or underlying neuroanatomy of the components of the VER (see [1, 19,

21]). The initial surface positive component (P1), occurring at about 18 msec in our cats, apparently represents activation of cortical elements while the subsequent negative component is thought to represent antidromic conduction along apical dendrites [3, 4, 7]. The secondary components, arriving after 50 msec (Fig. 1), are now believed to be influenced by unspecific reticular and thalamocortical pathways, although the course of these pathways is still a matter of debate [1, 13, 25, 26]. Based upon this differentiation of the components of the evoked response, various drugs have been described and classified in terms of their probable site of action. In general, amplitudes of all secondary evoked response components are decreased or disappear while latencies are increased by surgical anesthetic doses of barbiturates and inhalational anesthetics [6,8]. A number of CNS stimulants, including amphetamine, have been shown to produce a decrease in both amplitudes [12] and latencies [20] of primary and secondary components of the VER. The direct action of these agents on the midbrain reticular formation has been repeatedly demonstrated (for review see [20]).

In short, the restricted changes in the VERs of methadone-manic cats are not comparable to changes generally observed with those drugs which are presumed to involve the mesencephalic reticular formation as their primary site of action.

In some ways the effects of methadone in cats are consistent with the hypothesis that opioids inhibit the release of acetylcholine (ACH) from fibres originating in subcortical structures [17] and activating cholinoceptive neurones located in deep layers of cortex (see [18] for references). The fact that cortical application of an anticholinergic drug (atropine) reliably affects only secondary (>50 msec) components of the evoked response in cats [24] combined with the evidence that application of morphine to one hemisphere depresses the release of ACH bilaterally [16], suggests that opioid sensitive, subcortical neurones effect the secondary components (e.g., >50 msec) of the evoked response. However, we did not observe any significant methadone-induced alteration of the late negative wave (P3–N3) such as that observed in our monkeys. In short, methadone appears to have a restricted action in the visual system of the hyperexcited cat.

In summary, the results of the study suggest that methadone in the cat does not mimic the effects of those drugs, stimulatory or depressant, which are thought to affect primarily the midbrain reticular formation. There must be several levels of action of both methadone and its metabolites. This heterogeneity of sites and mechanisms probably changes with the time course of acute and chronic effects, the route of administration and assuredly with the species involved.

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